OUTBREAK OF A CHLAMYDOPHILA PSITTACI INFECTION IN LABORATORY RATS

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Abstract

During a study on Trypanosoma equiperdum propagation in adult male Wistar rats, four rats died spontaneously. Two of the dead animals were subjected to post mortem examination. From different organs of the rats, chlamydiae were isolated and confirmed by PCR and DNA sequencing as Chlamydophila psittaci. The results show that infection with Chlamydophila psittaci may occur in laboratory rats. Such outbreaks may have influence on the results of experimental studies. Chlamydial infections in laboratory animals also pose risk to humans (zoonosis).

Key words: rat, laboratory animals, Chlamydophila psittaci, PCR, DNA sequencing.

Chlamydia (C.) psittaci (formerly Chlamydia psittaci) is a Gram-negative obligate intracellular bacterium. C. psittaci is a member of the family Chlamydiaceae. This family has been reclassified recently and comprises now of two genera (Chlamydophila and Chlamydia) with nine species (4). Chlamydiae are widespread in birds and mammals. There are also some reports about Chlamydia psittaci in reptiles (12). Only few articles are published on chlamydial infections in rodents (2, 10, 11, 13). Yadava (13) described an infection of albino rats with high mortality caused by a psittacosis-like organism. The infected rats showed nasal discharge, staring coat, and loss of weight. The agent could be demonstrated in sections of the lung and liver. Damy et al., (2) reported a co-infection of rats with Mycoplasma pulmonis and Chlamydia pneumoniae. The agents were detected in colonies of laboratory rats by routine health surveillance. The rats showed increased levels of different serum enzymes and urea. Using electron microscopy, Chlamydia pneumoniae and Mycoplasma pulmonis could be detected in the lung, liver, spleen, heart, and kidney sections.

Here we report the isolation of C. psittaci from two adult male Wistar rats, which were used for a study on Trypanosoma (T.) equiperdum propagation. During this study, four out of nine rats died spontaneously; one of them even before the infection with T. equiperdum, and three of them after intraperitoneal injection of the parasite. Two of the latter animals were subjected to post mortem examination. Cell culture experiments were performed for the isolation of intracellular pathogens. From different organs of both of the rats, chlamydiae were isolated and confirmed by PCR and DNA sequencing as C. psittaci.

Material and Methods

Animals. A group of nine male Wistar rats reared under SPF conditions until they gained a body weight of 376-465 g, and were then transferred to a conventional environment for another six months. One rat expired at the end of this period; the remaining eight animals were injected intraperitoneally with live trypanosomes. The development of parasitaemia was controlled by phase contrast microscopy. Five animals exhibiting good parasitaemia were euthanised by cardiac puncture three days after the injection; one rat expired on the same day. Necropsy showed no macroscopically visible lesions in any of the euthanised animals. The remaining two rats, showing no parasitaemia, died five days later. A complete post mortem examination was carried out on these two animals. Smears from the heart, liver, spleen, kidney, and blood were prepared and stained with Giemsa stain for light microscopic examination.

Screening by cell culture. Isolation and cultivation experiments of intracellular agents were performed in BGM cells (6). The cells were originally obtained from FLI Insel Riems, Germany. Non-infected cell cultures served as negative control. Specimens that were positive by phase contrast microscopy underwent further characterisation. Cover slip cultures were infected with the agent isolated from the heart sample. The cover slips were fixed after an incubation period of 48 h and stained according to the method of Giménez (5) or with Giemsa stain.
PCR. Chlamydia-specific PCR was conducted according to the procedures of Everett and Andersen (3), Everett et al., (4), and Kaltenböck et al., (7), modified by Sachse and Hotzel (9), respectively. PCRs were carried out with tissue samples of the heart, liver, spleen, kidney, and blood and additionally with cell culture supernatants. Prior to the amplification, DNA was extracted from the samples using commercial kits (Puregene DNA Isolation Kit GW, Biozym, Hess., Germany, and High Pure PCR Template Preparation Kit, Roche Diagnostics, Germany). PCR products were separated by 2% agarose gel electrophoresis and visualised by ethidium bromide staining under UV light.

DNA sequencing. For DNA sequencing, bands were cut out of the gel and DNA was extracted using the QIAquick Gel Extraction Kit (QIAGEN, Germany). The extract was subjected to cycle sequencing using the BigDye™ Terminator Cycle Sequencing Ready Reaction Kit (Applied Biosystems, Germany) according to the manufacturer. The following oligonucleotides were used as sequencing primers: 16SIGF and 16SIGR for the 16S rRNA signature region (4), 201CHOMP and CHOMP336s for ompA gene section (9). Nucleotide sequences were determined using an ABI Prism 310 Genetic Analyzer (Applied Biosystems).

A comparison of the sequences with those from public database entries was carried out using the BLAST server (BLASTN 2.0.13, blast@ncbi.nlm.nih.gov).

Results

Pathological findings. One animal did not show macroscopically visible pathological lesions, the other one exhibited signs of splenomegaly, follicular hyperplasia, and serositis. Histological examinations of the organs were not performed. The Giemsa-stained cover slips of infected BGM cells showed inclusions, which were typical for chlamydiae. The intracellular growing bacteria could be detected by phase contrast microscopy 8-12 d after infection. Stained cover slips of infected BGM cells showed inclusions, which were typical for chlamydiae. The agent was identified as Chlamydia psittaci by the PCR according to Kaltenböck et al., (7) or as Chlamydyphila psittaci using the PCR described by Everett and Andersen (3). By PCR, Chlamydia psittaci could also be detected in the samples of the heart, liver, spleen, kidney, and blood. The result was confirmed by DNA sequencing of 16S rRNA signature region and part of the ompA gene. The analysed sequence of the ompA region was identical to that of Chlamydia psittaci strain 6BC (Acc. No. X56980) and C. psittaci strain MN Zhang (Acc. No. AF269281).

Discussion

Here we report a generalised infection with C. psittaci in laboratory rats, which occurred during a study on T. equiperdum propagation. The agent was isolated by cell culture and could be demonstrated by PCR in all tissues taken from two rats, which died during the experiment. Final identification was performed by DNA sequencing. Observations comparable to our findings were reported from Damy et al., (2), who detected chlamydiae in many organs of rats. The case reported by Yadava (13) may also be interpreted as a generalised infection of rats with a psittacosis-like agent.

In contrast to the unambiguous results reported here, several facts leading to the infection are uncertain. The source of infection and the route of transmission are unclear. Chlamydiae can be detected in apparently healthy animals, e.g. in mice where they seem to exist as commensals (13). Excreted chlamydiae can then be transmitted by aerosol. In order to check this hypothesis for the present case, five rats reared under SPF-like conditions in the same animal house were examined for chlamydiae by PCR and cell culture eight months later. However, all the samples gave negative results (data not shown). Therefore, the source of infection is still unknown. Additionally, the time point of the infection is not clear. Some rats introduced into the Trypanosoma experiment immediately after leaving the SPF conditioned animal house were free from chlamydiae. Therefore, the infection must have occurred sometime after transferring the animals to conventional environment. It might be possible that the experiments on T. equiperdum have then activated a latent infection with the agent. On the other hand, the presence of chlamydiae may have triggered the symptoms caused by the experimental infection with T. equiperdum. In cases mentioned by other authors, the laboratory animals showed co-infection with other pathogens, e.g. with Aspergillus sp. and Pasteurella multocida (13), Mycoplasma pneumoniae (2), or Streptococcus pneumoniae and Bordetella bronchiseptica (10).

From our findings, a conclusion can be drawn that C. psittaci infections can occur in laboratory rats. Such infections may influence the experimental results. Furthermore, infections with C. psittaci pose risk to the personnel in animal houses (zoonosis) (1, 8). Therefore, routine health surveillance in laboratory rodents should also include examination on chlamydiae. Additionally, in cases of nasal discharge, pneumonia, or sudden death in laboratory rodents, examination on chlamydiae should be performed. Cell culture and PCR may be the suitable tools for this examination.

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References


